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Characterization of shock in a hamster model of hantavirus infection

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Abstract

Human hantavirus cardiopulmonary syndrome (HCPS) due to Andes, sin nombre and other hantaviruses is characterized by severe pulmonary capillary leak and cardiogenic shock. Hamsters, the only animal manifesting HCPS-like disease, were instrumented with radiotelemeters that enabled ambulatory intracarotid blood pressure recording within an animal biosafety level-4 facility. Following infection with Andes virus, blood pressure and heart rate decreased slowly in a biphasic manner during the first 7 days of infection, followed by a rapid fall in pressure and rapid increase in heart rate during the 10–20 h preceding death on day 9 or 10. The preterminal narrowing of pulse pressure was consistent with a cardiogenic impairment. Heart rate variability analysis implicated increased sympathetic nervous system activity as seen in human HCPS. The hamster model of HCPS mimics not only the pulmonary capillary leak but also the hypotension characteristic of human HCPS.

Keywords: Hantavirus; Shock; Radiotelemetry; Hamster; Andes virus

Introduction

Hantavirus cardiopulmonary syndrome (HCPS), first recognized in the United States in 1993 (Duchin et al., 1994), is frequently complicated by not only massive pulmonary edema but also shock (Koster and Hjelle, 2004). A cardiogenic component to the shock is suggested by ventricular wall motion abnormalities, as recorded on two-dimensional echocardiography; reduced ejection fractions and stroke work despite normal filling pressures; and normal or elevated systemic vascular resistance (Hallin et al., 1996). Current treatment is limited to supportive care, vasopressor and inotropic medications, and extracorporeal membrane oxygenation where available (Crowley et al., 1998). Infection of the Syrian golden hamster with the Andes virus (ANDV) (Hooper et al., 2001) and Maporal virus (Milazzo et al., 2002) causes an illness that mimics human disease with respect to severe pulmonary edema and large pleural effusions. Whether the infected hamster also manifests shock, be it of cardiogenic, hemorrhagic, or circulatory origin, is not known.

The biosafety level-4 (BSL-4) classification of ANDV when used in hamsters limits the potential techniques for assessing cardiac depression, as most methods for determining contractility and cardiac index are fairly invasive. We implanted radiotelemetry devices prior to infection to continuously acquire blood pressure and heart rate data in conscious, unrestrained animals. Beat-to-beat interval data were analyzed for heart rate variability patterns using frequency domain techniques to indirectly assess the cardiac autonomic balance throughout the course of disease. We confirmed that significant shock precedes death in the hamster model of ANDV infection.

Results and discussion

Infection and mortality

Consistent with the original model description (Hooper et al., 2001), ANDV was highly lethal and all hamsters infected with ANDV died between days 9 and 10 of infection. Overt signs of infection were not apparent until 24 to 36 h prior to death and were manifested as hunched posture, ruffled fur, mattering and crusting around the eyes, and paucity of movement. Blood pressure telemetry permitted an estimate of diurnal patterns of

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activity and, while not quantitative, a decrease in activity was observed during the usual nocturnal hours beginning at least 48 h prior to death. Within 12 h of death, most hamsters demonstrated marked tachypnea. These clinical observations and the observed 100% mortality were similar to those previously reported (Hooper et al., 2001) although the earlier interval to death (days 9 and 10) might be due to infection of younger animals (10 weeks old) compared to death at days 11 to 14 in 14- to16-week-old hamsters.

Postmortem examination of the hamsters revealed a weight loss of approximately 5 g and large volumes (approximately 3 ml) of yellow to pink opaque fluid bilaterally in the pleural cavity. The heart appeared normal in size and in coloration; the pericardial cavity of one animal contained a small amount of fluid.

Blood samples collected from the hamsters at necropsy were tested for antibody (IgG) against ANDV, using an indirect fluorescent antibody test. The anti-ANDV antibody titers in all the virus-inoculated animals at necropsy were >40. Infectious hantavirus was isolated from samples of lung tissue from all the animals.

Hemodynamic measurements

Recordings of individual carotid arterial blood pressures exhibited a diurnal pattern varying with physical activity within the cage; periods of high activity often rendered inadequate signals due to artifactual noise. Averages of all of data from the recordings, minus the periods with uninterpretable signals, however, revealed clear trends for the group of 10 animals during the course of infection. During the first 3 days of infection following inoculation, blood pressure and heart rate decreased as did baseline systolic and diastolic blood pressures of 106 and 88 mm Hg, respectively, decreasing to 85 systolic and 72 diastolic within 72 h (Fig. 1A). The explanation for this decrease is not apparent and it may simply reflect accommodation to the environment, although the value is lower than expected normal values (Kato et al., 2003). During days 3 to 6 of infection the blood pressures appeared to stabilize, but following day 6, blood pressure began a second modest decrease until 10 to 20 h preceding mortality. Heart rates behaved similarly over the first 3 days and appeared to stabilize on day 3 but began a second phase of decrease from day 5 to roughly 10 h before death (Fig. 1B). Heart rates eventually fell to nearly half of the baseline values; again, this may be due, in part, to accommodation to the environment along with the decreased physical activity associated with the illness.

During the 12 h preceding death, blood pressure fell more rapidly (Fig. 2A) with systolic pressures falling below 75 mm Hg and diastolic pressures below 65 mm Hg, which is consistent with shock. Systolic pressure declined more rapidly than diastolic, resulting in a decrease in pulse pressure from roughly 9 mm Hg to 4 mm Hg. Heart rate increased during the final 12-h interval, increasing 20% before asystole (Fig. 2B). During the 1 to 2 h prior to death, agonal breathing patterns were observed in the blood pressure tracing.

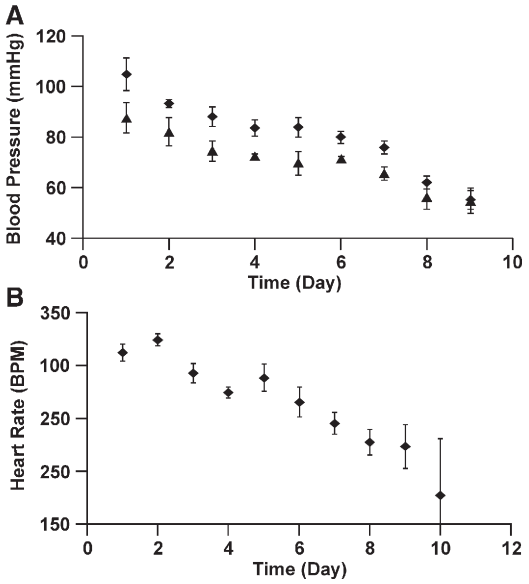

Fig. 1. Blood pressure (A) and heart rate (B) alterations over the course of ANDV-induced illness. Systolic (diamonds) and diastolic (triangles) data are shown as mean ± SE for all animals during each 24-h interval. Based on a paired ANOVA, significant decreases in blood pressure and heart rate were seen at day 4 compared to baseline, and on day 8 compared to day 4 ($p<0.01$). Variation in heart rates among the animals on the day of death (days 9 and 10) was considerable, owing to the dramatic hypotension and baroreceptor-related compensation.

The findings of decreasing pulse pressure are consistent with cardiogenic shock, but measurement of blood pressure in the absence of an independent measure of myocardial contractility and stroke work do not enable firm conclusions regarding the contribution of cardiac dysfunction to the hypotension. Other causes of shock in this disease may include (1) intravascular blood volume depletion due to capillary leak, which would be consistent with the observed weight loss; (2) mechanical interference by pulmonary edema and pleural effusions that negatively impact diastolic filling and, therefore, cardiac output; and (3) peripheral dilatation related to severe hypoxemia.

Heart rate variability

Heart rate variability (HRV) is the measurement of variations in the beat-to-beat intervals. HRV is normally somewhat chaotic but the intervals become more regular during disease. Complex mathematical analysis of interval variation yields quantitative markers of autonomic activity. These markers may reflect changes in peripheral vascular resistance that are difficult to measure in ambulatory animals. The dimensionless parameters correlating with the physiologic rhythms embedded in the intrabeat variations have been experimentally verified by a number of investigators, and summarized and standardized by a Task Force (Task Force, 1996). High frequency (HF) and root mean sum of squared deviations (RMSSD) are indices of rapid heart rate change principally affected by parasympathetic controllers. Low

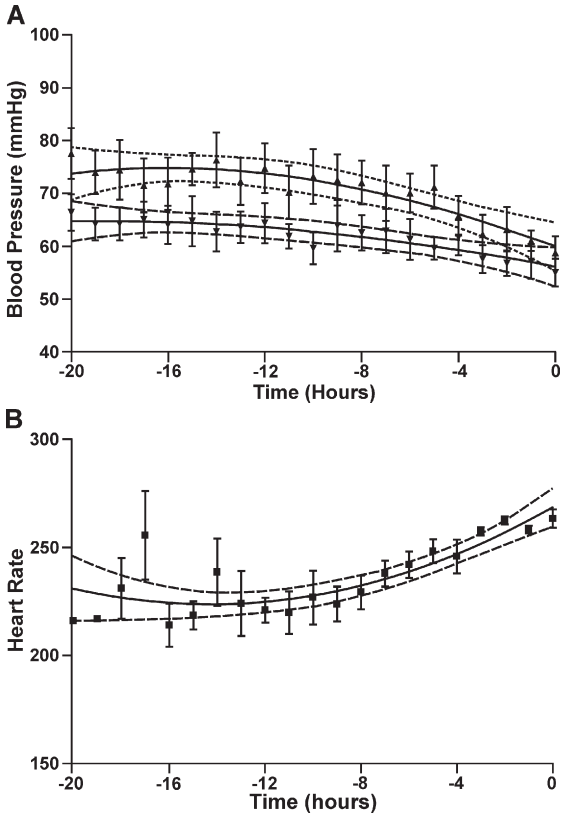

Fig. 2. Blood pressure (A) and heart rate (B) during the final 20 h of disease course, with the hour of death for each animal aligned at 0 h. Each hourly timepoint is the group mean (standard deviation) of the hourly means for each animal. Systolic pressure data (up triangle) and diastolic pressure (down triangle) in panel A, and heart rate in panel B, is shown with regression trends (solid lines) with 95% confidence interval (broken lines).

frequency (LF) and total power (TP) represent contributions from complex systems including thermoregulation and physical activity. The ratio LF/HF and the standard deviation of normal intervals (SDNN) are indices associated with medium- to long-term heart rate changes associated with sympathetic activity.

Similar to the blood pressure and heart rate patterns, HRV parameters displayed a biphasic trend over the course of the illness (Table 1). The LF/HF ratio, a nonspecific indicator of sympathetic nervous system tone to the periphery (Campen et al., 2005), increased significantly over the first 5 days but then reached a plateau. HF, TP, and rMSSD values fell consistently over the 10-day illness.

These findings are similar to HRV changes previously reported in septic humans (Annane et al., 1999), showing a decrease in TP and an increase in the LF/HF ratio. Other studies of circulatory shock, whether by sepsis (Ellenby et al., 2001) or hemorrhage, also show an increased LF/HF ratio. However, in the present study, the change in the LF/HF ratio was largely driven by a significant HF power decrease, with no changes in LF power.

Our previous examinations of HRV in rodents suggest that the LF/HF ratio reflects sympathetic discharge to the vasculature, with indirect effects on the heart as a result of cyclic baroreceptor modulation of systemic arterial blood pressure changes (Campen et al., 2005). Therefore, the increase in LF/HF power may implicate oscillations in vascular tone owing to the competitive feedback process of the baroreceptors, which would also affect oscillating discharges to the cardiac pacemakers. The decrease in HF may also relate to the lower tidal volumes observed during the latter stages of disease. The depth of breathing is directly related to the respiratory sinus arrhythmia, a result of both stretch receptor discharge and pleural pressure swings that mechanically impact on venous return.

Radiotelemetry measurements in high-level containment facilities

An important outcome of this study is the successful use of radiotelemetry in rodents in a BSL-4 laboratory facility. The design of the facility permitted set up of the master acquisition/analysis computer in a control area outside that containment space and thus did not require the donning of personal protective equipment, thereby facilitating remote monitoring and data acquisition. The development of techniques to better characterize morbidity in rodent models of infectious disease may enhance studies of drug efficacy. Humans with severe HCPS are commonly supported by oxygen, artificial ventilation, cardiopulmonary bypass, and a wide cocktail of circulatory support medications based largely on information derived from vital signs. On the other hand, animal models are generally described by survival percentage and duration with little data on physiological function, limiting the potential value of the animal model. Characterizing the disease pathophysiology, by telemetric or related methods, in the containment laboratories should provide important translatable information for animal models of human disease.

In conclusion, Andes virus infection in hamsters induces preterminal hypotension similar to shock as documented in humans with HCPS. While further characterization of the cardiopulmonary sequelae is required, this model may be useful for testing efficacy of novel interventions directed at late-stage shock in HCPS.

Table 1
Heart rate variability changes from the day of inoculation (day 1) until day 9

Parameter	Day			
	Day 1	Day 3	Day 7	Day 9
LF	0.96±0.04	0.94±0.02	0.92±0.02	0.90±0.08
HF	1.52±0.11	1.36±0.09*	1.16±0.04*	1.17±0.13*
LF/HF	0.67±0.04	0.75±0.05	0.83±0.05*	0.92±0.22*
TP	2.48±0.13	2.30±0.08	2.08±0.05*	2.08±0.08*
SDNN	0.079±0.008	0.068±0.008	0.081±0.007	0.062±0.004
RMSSD	0.137±0.023	0.088±0.010*	0.104±0.008*	0.082±0.004*

Abbreviations: LF=low frequency, HF=high frequency, LF/HF=ratio of LF over HF, TP=total power, SDNN=standard deviation of normal intervals, rMSSD=root mean sum of squared deviations. See text for explanation of parameters. Asterisks (*) indicate significant differences from Day 1 ($P<0.05$).

Materials and methods

Viruses

The original ANDV stock, strain Chile-9717869, was kindly provided by Pierre E. Rollin and Thomas G. Ksiazek (Special Pathogens Branch, Center for Disease Control and Prevention, Atlanta, GA) after four passages in Vero E6 cells. We (University of Texas Medical Branch [UTMB]) prepared a twice-plaque purified ANDV stock after one additional passage as previously described (Milazzo et al., 2002). The stock was passaged on Vero E6 cells and supernatant media were collected on day 6 of culture, aliquoted, and frozen until use in challenge experiments.

Animals

Syrian golden hamsters ($n=8$, male, 6 weeks old) were obtained from a commercial vendor (Harlan, Indianapolis, IN) and quarantined for 2 weeks in an Association for Assessment and Accreditation of Laboratory Animal Care-approved rodent housing facility (Lovelace Respiratory Research Institute [LRRI]). All procedures conducted were approved by the Animal Care and Use Committees of LRRI and the UTMB, respectively. Throughout the study, animals were housed individually and allowed food and water ad libitum. Housing was on a 12:12-h light/dark cycle.

Radiotelemetry

Animals were implanted with radiotelemetry devices (TA10PA-C20, DataSciences Intl., Arden Hills, MN) according to the manufacturer's recommendations. Briefly, midline incisions were made on the dorsal and ventral neck. The body of the telemeter was placed subcutaneously on the back, roughly between the scapulae, and sutured in place. The catheter was tunneled subcutaneously to the ventral incision. The carotid artery was temporarily ligated with 5-0 silk suture, an incision was made in the artery between the ligations, and the catheter was introduced through this incision. The catheter was then secured with suture and cyanoacrylate glue, and all dermal incisions were closed with interrupted silk suture reinforced with cyanoacrylate glue. Animals were allowed 7 days to recover from surgery prior to air transport to the UTMB BSL-4 facility.

Study protocol

After shipping and a 5-day quarantine, hamsters were housed in individual microisolators (cages) in the BSL-4 facility. Hamsters (now 10 weeks old) were infected by intramuscular (caudal thigh) inoculation with 2.0 $\log_{10}$ PFU Andes virus diluted in 0.2 ml phosphate-buffered saline. Telemetry recordings began immediately following inoculations. Animals were observed twice daily and telemetry data were continuously acquired until death. Moribund animals were euthanized when possible, but rapid deterioration prevented euthanasia prior to death in five animals. Necropsy was performed on all animals to retrieve the transducer/telemeter device and collect cardiac blood for antibody detection, and lung tissue for assay of infectious hantavirus.

Data acquisition

All blood pressure traces were continuously recorded digitally at 2000 Hz on an external hard drive. These traces were collected and analyzed in real time by a commercial software package (Gould Ponemah Life Science Suite, LDS, Valley View, OH) for systolic, diastolic and mean pressures, and heart rate. Using the same software, traces were replayed to extract beat-to-beat interval data for HRV assessment.

Data analysis

Beat-to-beat intervals were analyzed for frequency domain HRV parameters by a published method (Campen et al., 2005). All data were averaged by day and analyzed by analysis of variance (ANOVA) to establish group changes across the disease progression. One animal that died on day 7 was removed from the analysis. Probability values less than 0.05 (two-tailed) were considered significant.

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